CHEMICAL MANUFACTURERS ASSOCIATION

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February 15, 1999

Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
79 Alexander Drive, Room 3217
P.O. Box 12233
Research Triangle Park, NC 27709

Re: Call for Public Comments on Eleven Agents Proposed for Listing or Delisting From the Report on Carcinogens

Dear Dr. Jameson:

The Ethylene Oxide Industry Council (EOIC) of the Chemical Manufacturers Association (CMA) submits this letter and its attachments in response to the December 14, 1998 Federal Register notice requesting comment on the proposal of the National Toxicology Program (NTP) to upgrade ethylene oxide (EO) to the "known carcinogen" category.[1]

EOIC has recommended retention of the "reasonably anticipated" rather than "known" classification of EO: (1) the epidemiologic data are weak and inconclusive and do not support a known classification, and (2) the human population genotoxicity data are not predictive of carcinogenicity for purposes of hazard identification. Representatives from EOIC member companies (Dr. Jane Teta of Union Carbide and Dr. Ralph Gingell of Shell Chemical Company) and Dr. Preston of CIIT appeared at the Board of Scientific Counselors (BSC) meeting on December 3, 1998.

The BSC discussion on EO resulted in a sharply divided vote to upgrade (6-5, one abstention). The two reviewers, Dr. Belinsky and Dr. Yamasaki were also divided in their opinion as to the appropriate classification. (Dr. Belinsky favored a "reasonably anticipated" classification for EO, while Dr. Yamasaki supported upgrade).

[1] 63 Fed. Reg. 68783.

Rather than duplicate the information presented in EOIC's prior comments[2] and at the December 3 BSC meeting, EOIC wishes to address the principal arguments raised by the supporters of upgrade at the BSC meeting. EOIC continues to believe that upgrade is inappropriate. There appears to be consensus that EO epidemiologic data do not support upgrade. In contrast, the interpretation of EO human cytogenicity data was a controversial topic on which there was no consensus -- as evidenced by the split 6-5 vote of the BSC. Supporters of upgrade believe that EO chromosomal data should be presumed to be predictive of cancer based on the fact that for another chemical, benzene, chromosomal alterations were found to be transmissible using fluorescence in situ hybridization technique (FISH). Similar FISH studies have not been conducted with EO, and it is unknown if the same finding would result. Informed scientific hunch is not the same as data or evidence and does not justify upgrade to the known carcinogen category.

EOIC appreciates the opportunity to submit these comments. For further information, please contact Kathleen Roberts, Manager of EOIC, at 703-741-5613 or Kathleen_Roberts@cmahq.com (e-mail).

Sincerely yours,

Has Shah, for

Courtney M. Price
Vice President
CHEMSTAR

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2 EOIC has submitted two prior sets of comments on the topic to NTP, dated March 23, 1998, and November 20, 1998.

COMMENTS ON NATIONAL TOXICOLOGY PROGRAM PROPOSED CLASSIFICATION OF ETHYLENE OXIDE

63 Fed. Reg. 68783 (December 14, 1998)

SUBMITTED BY

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SUPPLEMENTAL COMMENTS OF THE ETHYLENE OXIDE INDUSTRY COUNCIL NATIONAL TOXICOLOGY PROGRAM PROPOSED CLASSIFICATION OF ETHYLENE OXIDE NINTH ANNUAL REPORT ON CARCINOGENS

EXECUTIVE SUMMARY

Ethylene oxide ("EO") is an animal carcinogen. The large epidemiologic data base indicates no increased risk of cancers overall or of brain, stomach or pancreatic cancers. The findings for certain cancers of the lymphopoietic tissues, specifically leukemia and non-Hodgkin's lymphoma ("NHL"), are inconclusive. Based on an overall assessment of the epidemiology, there is limited evidence in males but no indication of an excess risk for these diseases in females.

At the December 3, 1998 meeting of the Board of Scientific Counselors ("BSC") most of the discussion centered on the controversial question of the relevance of the genetic data and in particular human population monitoring studies. Both *in vitro* and *in vivo* studies of EO have detected positive responses for a number of genotoxic endpoints. These include point mutations, sister chromatid exchanges ("SCEs"), chromosomal aberrations, micronuclei, DNA adducts and hemoglobin adducts. The predictive relevance of any of these data to cancer hazard assessment has not been demonstrated. Currently available human cytogenetics monitoring data are of limited relevance to carcinogenicity in humans due to: 1) the inconsistency with the direct evidence from human cancer studies, 2) concerns about the lack of relevance of certain endpoints as indicators of adverse health outcomes, 3) the endpoints that might be assessed reflect only

recent, not past, exposure and 4) concerns about the small size of the human cytogenetic monitoring studies and the potential for confounding factors.

As Dr. Julian Preston's [CIIT] analysis shows[1]:

- The chromosome aberration types assessed in human cytogenetic monitoring studies are not those that are observed in human tumors, hematopoietic or other.
- Extrapolating from the aberration types observed for ethylene oxide exposed individuals, namely chromatid deletions, to the transmissible reciprocal translocations seen in tumors is not correct. Observing deletions does not imply that transmissible reciprocal translocations might be induced.
- Sister chromatid exchanges are not associated with mutagenic responses.
- Observations of genetic alterations in terminally differentiated peripheral lymphocytes cannot be used to predict tumor induction in other cell types.
- Human population monitoring studies as conducted to the present time can be used as predictors of exposure not of carcinogenicity.

Because the human population monitoring data on genotoxic effects of EO do not measure effects of past levels of exposure, but rather of recent exposures, and because the study designs are limited by small sample size and potential for confounding, EOIC has urged NTP that greater reliance for evidence of carcinogenicity should be placed on long-term, well conducted occupational mortality studies. The "known" classification is inappropriate.

I. The EO Human Cytogenetic Data Do Not Support Upgrade

A. The Endpoints Observed In Cytogenetic Studies Have Not Been Demonstrated To Be Relevant To Predicting Carcinogenicity

At the BSC meeting, Dr. Preston addressed the potential relevance of each of the endpoints associated with EO in the genetic toxicity studies:

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[1] **Preston, R.J., "Cytogenetic Effects of EO With an Emphasis on Population Monitoring" (1999) (submitted for publication)**; BSC Hearing Transcript ("Tr.") at 319-22.

- SCEs are biomarkers of recent exposure but are not considered relevant to prediction of cancer or any other disease outcome. SCEs are not associated with mutagenic responses.[2]

- Micronuclei are cell lethal events that are not involved in tumors because they are not transmissible.

- Peripheral lymphocytes are terminally differentiated cells that are not involved in tumor formation. Chromosomal changes in these cells are not relevant for predicting tumors in other tissues and organs. Observations of genetic alterations in terminally differentiated peripheral lymphocytes cannot be used to predict tumor induction in other cell types. Tr. 319-21.

Chromosomal aberrations observed *in vitro* in peripheral lymphocytes of humans do not indicate lasting chromosomal alterations. In contrast to *in vitro* data, EO is not a potent mutagen in vivo.[3] It is unlikely that one can show from *in vitro* experiments what amount of damage, induced over a period of time prior to taking a blood sample, would remain in the lymphocyte available to be converted into a lasting chromosomal alteration.[4] If human peripheral lymphocytes are treated *in vitro* with a very potent alkylating agent

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2 Preston, R.J. *et al.*, "Short/Medium Term Carcinogenicity Tests and Genetic and Related Effects," IARC Meeting - Lyon, France, October 1997 (In Press). See also Stolley, P.D., "Sister-chromatid exchanges in association with occupational exposure to ethylene oxide," Mutation Research 129: 89-102.

3 **Preston, R.J.,** "Cytogenetic Effects of EO With an Emphasis on Population Monitoring" ~~(1999)~~ **(submitted** for publication).

4 Id.

(triethylenemelamine), the DNA damage is sufficiently repaired within 48 hours such that chromosomal damage is not evident.[5] Dr. Preston observed:

> A peripheral lymphocyte is a noncycling cell. To produce the events that are looked at in the monitoring studies, one requires DNA synthesis. The study of the lymphocytes as normally conducted the S phase [occurs] in the test tube after the blood sample is taken. So in fact the genetic alterations that are looked at aren't produced in the human at all. They are produced in a test tube. So there are a number of events that can occur in the test tube that could also influence the outcome of the study.

Tr. 321.

Extrapolating from the aberration types observed in EO-exposed individuals -- chromatid deletions -- to transmissible types observed in humans is not correct. Observation of chromatid deletions does not imply that transmissible reciprocal translations might be induced.[6]

An additional limitation of EO genetic toxicity studies conducted in humans is their limited sample size and consequent inability to rule out potential confounding effects with a reasonable degree of confidence. Tr. 320-21. Dr. Preston discussed three population monitoring studies by Schulte, Ribeiro, and Tates to illustrate these points:

- Schulte *et al.*[7] suffered from limitations of small size, limited exposure data, presence of confounders, and an unusual statistical analysis. The values

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5 Id. (citing Bast and Preston) (unpublished)).

6 Brewen, J.G., Natarajan, A.T., and Obe, G., "Mutagenicity of selected chemicals in mammalian in vitro cytogenetic systems." In *Comparative Chemical Mutagenesis* (Eds. F.J. deSerres and M.D. Shelby) Plenum Press: New York, pp. 433-485 (1981).

7 Schulte, P.A. *et al.*, "Biologic markers in hospital workers exposed to low levels of ethylene oxide," Mutation Research 278: 237-251 (1992).

he found for SCEs in control groups were much lower than those observed in larger studies. The U.S. study group consisted of 8 unexposed controls, 32 in the lower exposure group and 11 in the higher exposure group. The Mexico study group consisted of 9 and 12 in the two exposure groups respectively, but only 1 control individual.

The SCE values for the two exposure groups of the U.S. study group, when corrected for confounding factors (age, sex, race, smoking, and tea consumption), were reported to be higher than the control value. The mean control value for the very small number of individuals that Schulte studied is significantly lower than obtained in larger, control population studies using very similar methods. Taking SCE values, Schulte's U.S. control group shows SCE mean values of 4.61 per cell. Other larger studies report SCE values of 8.29 ± 0.08 for 353 individuals,[8] and 9.32 for 22 non-smoking individuals.[9] Thus in Schulte *et al.,* the exposure group has higher than normal SCE measurements, but the control group has lower than normal SCE measurements, thus falsely suggesting an exposure-related effect.

The number of controls was much too small in the Schulte *et al.* study -- eight U.S. workers and one in the Mexican worker group. There is indication that the insufficient number of controls is not merely a formal deficiency, but may undermine Schulte's finding of a significant excess in SCE values and hematocrit values.

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8 Bender, M.A. *et al.*, "Chromosomal Aberration and Sister-Chromatid Exchange Frequencies in Peripheral Blood Lymphocytes of a Large Human Population Sample," Mutation Research 204, 421-433 (1988).

9 Tucker, J.D. *et al.*, "Variation in the Human Lymphocyte Sister-Chromatid Exchange Frequency: Results of a Long-Term Longitudinal Study," Mutation Research 204, 435, 444 (1988).

In addition, in the 1992 Schulte *et al.* study, the estimate of four months of cumulative exposure was based on only two to four days of EO measurements. Schulte *et al.* acknowledge as study "weaknesses" the fact that "the estimate of exposure was based on 2-4 days of EO measurements to model the cumulative exposure. The impact of peak exposures or other variations from the mean of those measurements could not be assessed." *Id.* at 249.

- Ribeiro *et al.'s* finding of increased SCEs may be attributable to the workers' exposure to other chemicals. The authors concluded that the relatively strong positive effects in their study, at relatively low TWA ethylene oxide concentrations could be "due to the possibility that the workers in Brazil were exposed not only to EtO but also to other chemicals used in the working process." Limited measurements of hemoglobin adducts tend to support this view. This concern is of particular importance in any population monitoring study, and must be able to be taken into account when using such studies for risk assessment purposes, i.e., both qualitatively and quantitatively.[10]

- The Tates study -- not available to IARC when it classified EO as Group 1 -- shows that if there is a significant time interval between exposure and sampling, there is no increase observed in chromosome aberrations. Tr. 321-22. Tates

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[10] Ribeiro, L.R., Salvadori, D.M.F., Rios, A.C.C., Costa, S.L., Tates, A.D., Tornqvist, M., and Natarajan, A.T., "Biological monitoring of workers occupationally exposed to ethylene oxide," Mutation Research 313: 81-87 (1994)

found no evidence of *hprt* mutations, SCEs, or micronuclei for highly-exposed workers sampled 89-180 days after exposure.[11]

In sum, human population monitoring studies as conducted to date can be used as predictors of exposure, not of carcinogenicity. Dr. Preston concludes that (i) the chromosomal aberrations measured in the EO studies are not correlated with transmissible events and cannot be used to predict human carcinogenesis; (ii) the studies as conducted are not appropriate for detecting long-term EO exposure, and (iii) endpoints observed at acute high concentrations are indicative only of recent exposure, not harbingers of adverse health outcomes. Tr. 322.

Dr. Preston addressed several points raised by those who supported upgrade:

- For benzene, it has been demonstrated that chromosomal alterations later develop into tumors.

As Dr. Preston pointed out (Tr. 323), in the benzene study transmissible alterations were identified using fluorescence *in situ* hybridization (FISH) techniques. Unless and until such studies are conducted in humans, allowing measurement of events induced in precursor cells that occur in humans (rather than the test tube) it will not be known whether EO induces heritable reciprocal translocations. Tr. 328.

- EO is genotoxic in all phyla studied, including in animals where chromosomal aberrations and tumors have been observed.

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[11] Tates, A.D., Grummt, T., Tornqvist, M., Farmer, P.B., van Dam, F.J., van Mossel, H., Schoemaker, H.M., Osterman-Golkar, S., Uebel, Ch., Tang, Y.S., Zwinderman, A.H., Natarajan, A.T., and Ehrenberg, L., "Biological and chemical monitoring of occupational exposure to ethylene oxide," Mutation Research 250: 483-497 (1991).

Under NTP criteria, there must be human mechanistic data, not merely animal mechanistic data, to support upgrade under NTP criteria. Tr. 316, 317. As Dr. Henry pointed out, the chromosomal aberrations and SCEs observed for EO "are definitely reflections of exposure. In animals they have been shown to be correlated with tumor production, but the mechanistic steps between that in people and tumor production is not known." Tr. 327.

The mechanistic study by Tates (1995) (*supra*) involved human exposure to EO at levels of 52 to 785 mg/m^3, and found no increase in genetic endpoints for workers sampled several months after exposure. Thus the Tates study does not support reliance on cytogenetic data to upgrade EO. Tr. 322, Tr. 369.

- One small study, by Hogstedt,[12] found micronuclei formation in bone marrow cells -- recycling cells.

As Dr. Preston pointed out at the BSC hearing, and in his paper,[13] the study by Hogstedt is a very small scale study and does not provide supportive evidence for induction of chromosome aberrations.[14] *Id.* at 319. In Hogstedt, the frequencies of SCE and micronuclei were not increased in the exposed groups. The frequency of chromosome aberrations was not elevated in bone marrow cells of the exposed group,

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12 Hogstedt, B., Gullberg, B., Hedner, K., Kolnig, A.M., Mitelman, F., Skerfving, S., and Widegren, B., "Chromosome aberrations and micronuclei in bone marrow cells and peripheral blood lymphocytes in humans exposed to ethylene oxide," Hereditas 98: 105-113 (1983).

13 Preston, R.J., "Cytogenetic Effects of Ethylene Oxide With An Emphasis on Population Monitoring" (1999) (submitted for publication).

14 Dr. Yamasaki recognized that the relevance of this study was a "judgmental issue" where some might not judge "as strong." Tr. 318.

although there was the suggestion of an increase of micronuclei in erythroblasts and polychromatic erythrocytes. These data are difficult to interpret and should not be used as an unequivocal demonstration of a clastogenic effect of ethylene oxide in exposed persons, especially given that SCEs, the more sensitive endpoint for genotoxic chemicals, showed no increase in the exposed groups. Hogstedt's small study does not support a "known" classification under NTP's criteria, which require "sufficient evidence of carcinogenicity from studies in humans that indicates a causal relationship between exposure to the agent, substance or mixture and human cases."[15]

Our understanding of human mechanistic data is evolving. Interpretation of the significance of EO human cytogenetic monitoring data is controversial, as evidenced by the 6-5 split vote of the BSC. As our understanding increases, it may be necessary to re-evaluate EO's carcinogenicity potential. Under the current state of knowledge, upgrade would be inappropriate and could require early reconsideration of EO's classification as additional knowledge is acquired.

II. The EO Epidemiologic Experience Calls Into Question Reliance On Cytogenetic Data For Hazard Identification

EOIC submits that NTP's criteria for "known carcinogen" are not met for EO. While the animal carcinogenicity data are sufficient, the epidemiologic evidence is weak and inconclusive and the genetic toxicity data are of uncertain relevance, reliability and validity. There is sufficient evidence that EO causes increased tumors at multiple sites in male and female rats and mice. The relevance of these findings to humans is uncertain because of the unconvincing evidence of carcinogenicity in a large body of well conducted, long-term studies of

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15 NTP Classification Criteria, Report in Carcinogens, 8 ed.

workers exposed to EO at high levels of exposure that prevailed in the past. The epidemiology is in contrast with the human genetic toxicity studies reporting measurable effects at low current workplace exposure levels.

The value of having a large data base -- 10 distinct epidemiologic cohorts -- is the ability to assess the overall consistency of findings with those from isolated human studies and animal bioassays. The extensive EO epidemiologic data base includes nearly 33,000 male and female EO workers with more than 800 cancers from five countries. Many of the studies have extensive follow-up, from the early years of the industry. For the occupational EO studies, average duration of exposure was up to 10 years with many workers having experienced 20 or more years of EO exposure, commencing in the early years of the industry. Average follow-up periods ranged from 7-28 years, with a maximum of 53 years. The largest of these studies is the NIOSH study by Steenland *et al.* of 18,254 male and female workers in 14 plants sterilizing medical devices and spices.[16] This study has an average observation period of 16 years with approximately 4,900 workers (28%) followed up for more than 20 years from first exposure. In the published version of the study, it is stated that 8% of the cohort had more than 20 years from first exposure. Drs. Steenland and Stayner have recently advised EOIC that "8%" was an error in their paper and that "28%" is the correct percentage of workers with more than 20 years from first exposure.[17]

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16 Steenland, K., Stayner, L., Greife, A., Halperin, W., Hayes, R., Hornung, R., and Nowlin, S., "Mortality Among Workers Exposed to Ethylene Oxide," N. Eng. J. Med. 324, 1402-07 (1991).

17 Personal communication from K. Steenland and L. Stayner to J. Teta dated February 9, 1999 on file with Dr. Teta.

The EO epidemiologic data base is unusually rich. An objective review of the updated EO meta-analysis and tests of heterogeneity provide compelling evidence that the putative high risk for leukemia based on the early Hogstedt reports was an incorrect inference. Other studies of worker populations using or producing EO during the infancy of the chemical industry (when exposures were high) show no increase in leukemia. There are data from both animal and human studies suggesting that certain cancers of the lymphohematopoietic tissues (leukemia, NHL) warrant additional epidemiologic study. For some chemicals, a failure to detect excess cancer may be attributable to a sparse data base or limited follow-up, but that consideration does not apply to EO.

EOIC urges recognition that all published studies constitute the underlying data cited by Dr. Preston and Dr. Teta. In addition, Dr. Preston's 1999 review, which provides additional interpretation, has been submitted for publication, and Dr. Teta's 1999 article updating Roy Shore's meta-analysis is expected to receive final acceptance for publication within a matter of weeks.

CONCLUSION

NTP should retain the "reasonably anticipated" carcinogen classification for EO.